AD_____

Award Number: DAMD17-98-1-8470

TITLE: The Effect of eIF4E Antisense RNA Expression on Prostate Tumor Angiogenesis and Growth

PRINCIPAL INVESTIGATOR: Briana J. Williams, Ph.D.

CONTRACTING ORGANIZATION: Louisiana State University Medical Center
Shreveport, Louisiana 71130-3932

REPORT DATE: September 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1999	Annual (1 Sep 98 - 31 Aug 99)

4. TITLE AND SUBTITLE
The Effect of eIF4E Antisense RNA Expression on Prostate Tumor Angiogenesis and Growth

5. FUNDING NUMBERS
DAMD17-98-1-8470

6. AUTHOR(S)
Briana J. Williams, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Louisiana State University Medical Center
Shreveport, Louisiana 71130-3932

E-MAIL:
bwilli4@lsumc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

Some mRNA with complex 5' regions that are more efficiently translated in the presence of high levels of the translation initiation factor eIF4E are particularly important to the initiation and progression of prostate cancer, such as vascular enothelial growth factor (VEGF), basic fibroblast growth factor (FGF-2), and c-myc. The purpose of our study was to investigate the ability of an eIF4E antisense RNA approach to regulate the expression of VEGF and FGF-2, and thus tumor angiogenesis, and to better define the interactions between eIF4E and c-myc or p53 (which we suspect may be regulated by eIF4E as well) in prostate cancer. Each of 6 prostate tumor cell lines stably transduced with a retrovirus-mediated eIF4E antisense (4EAS) make less VEGF, FGF-2, and are significantly less able to recruit vascular cells or to stimulate either proliferation or differentiation into capillary-like structures than the parental cells. Suppression of eIF4E did not appear to influence the isoforms of FGF-2 expressed, but did appear to alter the relative abundance of particular VEGF isoforms. Animals injected with 4EAS transduced cells did not develop tumors compared with animals injected with parental cells, and 4EAS-seeded sponges were infiltrated with significantly fewer new blood vessels than sponges seeded with parental cells.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Prostate Cancer	22
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

___ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Briana J Williams 2-9-00

PI - Signature Date

TABLE OF CONTENTS

INTRODUCTION

The purpose of this study was to investigate the role of the translation initiation factor eIF4E in prostate tumor angiogenesis and to determine if suppression of eIF4E might be developed as a useful gene therapy. The specific aims included generation of cell line models that either overexpressed eIF4E or were suppressed for eIF4E to determine the effect of eIF4E levels on cell growth and an angiogenic phenotype when the cells were grown in animals. The cell lines were chosen to reflect varying p53 and c-myc levels in order to test the relationship between eIF4E and these genes in control of cell growth or the generation of the angiogenic phenotype. These genes were chosen because eIF4E is known to regulate, through a positive feedback mechanism, c-myc expression and p53 is appears to act as an anti-angiogenic gene, through control of both the pro-angiogenic vascular endothelial growth factor (VEGF) and the anti-angiogenic thrombospondin-1 genes [1-3]. Since p53 is frequently lost in prostate cancer progression and eIF4E expression is increased, the relationship between these 2 genes and their influence on tumor angiogenesis is interesting. Our preliminary studies of human prostate tumors suggested that elevation of eIF4E was frequent event and that eIF4E elevation correlated with overexpression of VEGF, which has been reported to be correlated with poor outcome in prostate cancer [4, 5]. We have measured eIF4E levels in an expanded collection cell lines with varying levels of p53 as well as generated sublines overexpressing eIF4E and carrying an eIF4E antisense construct to suppress eIF4E. We have tested these sublines for the effect of eIF4E on expression of both VEGF and FGF-2, another potent angiogenic growth factor. To date, some of the sublines have been grown in nude mice to investigate the role of eIF4E in vivo on tumor growth and angiogenesis.

BODY

Task 1: Construction of the retroviral vectors and purification of virus.
Timeline: Approximately 4 months to construct and package and purify the viruses.
Milestones: Measurement of eIF4E protein by western blotting to test for either increased expression (sense) or suppressed expression (antisense).
Deliverables: Purified retroviruses with a titer of at least 5 x 10^7 p.f.u. and have different measurable levels of eIF4E by western blotting.

Progress report for Task 1:

The retrovirus was purified and used for infection studies, although we were unable to purify the virus to the desired titer (as discussed below). PC3 and DU145 cells were infected with eIF4E sense (4ES) and antisense (4EAS) engineered virus and generated as stable lines under G418 selection. The levels of eIF4E were approximately 2-4 fold higher in 4ES cells, and approximately 50% of parental levels in 4EAS cells for both cell lines. **We chose to focus on cell lines which can be grown on plastic for year 1, and will begin studies on the LuCaP 23.1 tumor cells (which are passaged through mice) in year 2.**

Task 1 problems:
We have passaged the viral construct through 2 different packaging cell lines without increasing our titer. The retroviral titers we can achieve at present are sufficient, though not practical, for *in vitro* studies. Unfortunately, the infection frequency is so low that the resultant stable cell lines are not representative of the heterogeneous parental cell line and the time required to re-grow cells is quite long. More importantly, the infectivity is too low for practical use in *in vivo* studies, as we cannot reliably deliver enough virus to modulate the tumor growth we would expect from our *in vitro* results. For these reasons, we have chosen to develop other viral and non-viral approaches.

New approaches under development:
1. We have established a collaboration with William Jia at The Prostate Centre in Vancouver, British Columbia, to generate a herpes virus-mediated 4EAS viral construct. We believe this approach is particularly attractive as herpes virus appears to have a natural affinity for the prostate and will provide the advantages of genomic integration and infection of non-dividing cells. Our preliminary studies suggest that we can generate levels of eIF4E suppression significantly greater than those we obtained from the retrovirus. A more comprehensive evaluation will be presented in the next annual report.

2. We also have used the same 4EAS construct centered over the translation start site in both episomal and integrating plasmids. The episomal plasmid was not particularly stable even under stringent selection and did not consistently given the expected differential in 4E expression. It was therefore abandoned for our studies. The integrating plasmid is based on the pBK-CMV backbone and has performed very well in our system. It was used in most of our continuing in vitro experiments as it provided the highest level of differential 4E expression and is stable over many population doublings. The

3. We are developing use of a wholly synthetic antisense oligo, which also binds across the translation start site of the eIF4E mRNA, built of morpholine (rather than deoxyribose sugar) bases as a non-viral alternative to 4EAS delivery. To date, the results are very encouraging. These transfected cells have long term expression of 4EAS activity, and significantly decrease the levels of VEGF and FGF-2 in our system. An abstract outlining our success

with this oligo was accepted for presentation at a meeting to be held in early 2000 as described in the "Reportable Outcomes" section. In brief, we have been able to increase the suppression of eIF4E to 300% relative to parental cells, compared with 50% suppression we obtained with the retrovirus. In addition, the morpholine oligo suppressed growth of PC3 cells in a dose-dependent manner resulting in a maximal 10-fold suppression of growth. Finally, we tested the effect of this antisense eIF4E oligo on secreted VEGF production from PC3 cells and found that again this construct is significantly better able to suppress VEGF secretion, reducing VEGF levels more than 10-fold. This is more than 3 times the maximal suppression we obtained with the retrovirus or integrating plasmid constructs. We believe the morpholine construct binds with a higher affinity to the eIF4E mRNA and since it is resistant to endonuclease degradation, it has a longer "useful life" in suppressing eIF4E and ultimately VEGF. Our future goals are to develop a prostate tumor cell-specific targeting of the morpholine oligo through coupling with the folate receptor and to develop in vivo testing of this oligo. These studies will be presented more fully in next year's annual report.

Task 2: Infection of cell lines and growth assays.
Timeline: Approximately 2 months to test all cell lines for growth using the crystal violet growth assay and soft agar cloning.
Milestones: Preliminary data which will reflect the cell lines potential for tumorigenicity.

Progress report for Task 2:

Stably transduced cells from the retrovirus infection, the pBK-CMV plasmid, and the morpholine oligo have been tested for changes in cell growth. We have also expanded the number of cell lines to include those with wild type (PZ-HPV-7), null (PC3), and mutant p53 (DU145--we previously incorrectly thought them to be wild-type for p53), androgen sensitive/responsive (LNCaP and ALVA 101) and resistant (PPC-1, PC3, and DU145) cell lines. The sense expressing cells all grew at accelerated rates compared with parental or empty vector transduced cells, while the 4EAS expressing cells grew more slowly than parental or empty vector transduced cells, as illustrated in **Table 1**. Transduced cells also were grown in soft agar to test changes in contact independent growth. Cells were plated in 0.6% agarose (SeaPlaque, FMC, Inc.) in duplicate wells of 6 well dishes at a density of 1 x 10^3 cells and numbers of colonies >50 cells were counted after approximately 21 days. Parental and empty vector transduced cells formed approximately the same number of colonies and were not significantly different from the number of colonies formed by the 4ES cells. The suppression of 4E in all cell lines, other than PZ-HPV-7, resulted in the formation of fewer colonies, as illustrated in **Table 2**.

Interestingly, we did not see large differences in the effect of eIF4E (either overexpression or suppression) relative to p53 status. It appears from our results that the effects of eIF4E on cell growth are through p53-independent pathways supporting our hypothesis that eIF4E is a viable target for growth suppression in a wide variety of genetic backgrounds and will therefore be potentially useful as a gene therapy for a broad spectrum of patients.

Not completed:
All work with LuCaP 23.1 cell line, which will begin in year 2.

Task 3: Test for utilization of FGF-2 alternative translation start sites.
Timeline: Approximately 2 months to complete testing of all cell lines for FGF-2 activity using the HUVEC assay.
Milestones: Completion of the HUVEC assay

Progress report for Task 3:

We have completed the HUVEC assays using either co-culture or conditioned media collected from PC3, DU145, ALVA 101, PPC-1, and PZ-HPV-7 cells. In all cases, 4ES transduced cells stimulated significantly more migration and differentiation of HUVEC cells than the parental or empty vector transduced cells, as illustrated in PC3 cells in **Figure 1**. In addition, suppression of eIF4E using any of our constructs significantly blocked the differentiation of the HUVEC into capillary-like structures as illustrated in the lower right panel of **Figure 1**. In this assay we used fresh human umbilical vein endothelial cells obtained from our Department of Physiology tissue culture core facility that were cultured in the presence or absence of conditioned media collected from each of the parental cells and their cognate sublines. After 2-5 days in culture (depending on the cell line and the construct used), we saw development of capillary-like structures suggestive of endothelial cell migration and differentiation. In addition, we found that eIF4E stimulated proliferation of HUVEC and eIF4E antisense constructs significantly suppressed proliferation of HUVEC as illustrated using PC3 cells in **Figure 2**. We had originally thought this assay to be dependent on FGF-2 levels, but have found this to be incorrect. Using neutralizing antibodies, we have blocked FGF-2 and VEGF either independently or in combination and found that any of these conditions significantly decreases the 4ES mediated increase in migration and differentiation ($p< 0.001$), although none completely abolished migration or differentiation. These results suggest both VEGF and FGF-2, as well as other angiogenic factors, are responsible for the migration and differentiation and 4ES may mediate the increases in any of the factors.

New approaches completed:

The general focus of our studies has been on translational control of mRNA from certain angiogenic genes, particularly VEGF and FGF-2, but we also acknowledge that there may be many more such mRNA which could be equally important to the induction or maintenance of the tumor angiogenic phenotype. These mRNA can (and should be) identified using cDNA array technologies. In addition, eIF4E may be responsible for more indirect changes in gene expression by translating transcription factors that would alter angiogenic gene expression as a down-stream event responsive to changes in eIF4E levels. We used commercially-available cDNA expression arrays to identify other candidate angiogenic genes affected by alterations in eIF4E, and in our preliminary studies using the PC3 prostate cancer cell line, we found that in addition to translational control over VEGF and FGF-2, alterations in eIF4E levels resulted in significant changes in mRNA for TIE-1 (an angiogenic receptor gene with no known ligand) , Kdr (VEGF-2 receptor), and VEGF-C, which were all significantly up-regulated in 4ES and significantly down-regulated in 4EAS cells. These have now become interesting candidates as eIF4E-responsive genes in prostate tumors we are exploring their role in promoting or maintaining the angiogenic phenotype.

Task 4: Western blotting to test modulation of VEGF and FGF-2 translation by antisense eIF4E RNA.
Timeline: Approximately 2 months to complete testing of all cell lines for VEGF and FGF-2 protein.
Milestones: Completion of *in vitro* cell assays.
Deliverables: A manuscript outlining the effect of retrovirus-mediated eIF4E antisense RNA on cell growth and VEGF and FGF-2 expression.

Progress report for Task 4:

In a more direct measure of FGF-2 alternative start site utilization, we have examined by western blot the pattern of FGF-2 expression in parental, vector only, 4ES and 4EAS cells. **Appendix, Figure 3** illustrates the eIF4E levels, and the VEGF and FGF-2 patterns seen in each of the parental cell lines. While some of the cell lines had distinctive patterns of expression, we did not see any changes in either VEGF or FGF-2 isoform useage in response to eIF4E levels. We, therefore, have no evidence that 4E modulates the same changes in FGF-2 seen in from previous studies of the MDA-435 breast cancer cell line [6], and this may simply reflect the difference between the biology of the two tissues or variability between cell lines.

We have seen, however, evidence for an apparent relationship between eIF4E and its effect on FGF-2 and VEGF expression in prostate cell lines that appears to be unrelated to an "isoform switching" mechanism. As illustrated in **Figure 3**, different cell lines have differing levels of total FGF-2 protein, as well as different relative levels of the 22kDa and 18kDa isoforms. The western blot illustrated in this figure was made from whole cell lysates of each cell line, therefore a reflection of secreted and non-secreted forms of the protein. We calculated the amounts of each isoform relative to the β1 tubulin subunit as a loading control and saw interesting patterns emerge. Although DU145 appears to have significantly more of all FGF-2 isoforms than the other cell lines, its eIF4E levels are quite low relative to the other cell lines. In fact, from ELISA assays we know that cultured DU145 cells secrete very little FGF-2 into its surrounding media as presented in **Table 3**. On the other hand, ALVA 101 cells secrete significantly more FGF-2 into the media than any of the other cell lines, $p<0.001$. Given that the eIF4E levels in ALVA are relative high, we suggest that eIF4E may control, by some unknown and presumably indirect mechanism, the secretion of certain FGF-2 isoforms without "switching" predominance of the isoform in total protein. The immortalized "normal" cell line, PZ-HPV-7 had relatively low levels of eIF4E and very low levels of both FGF-2 and VEGF. This cell line may be particularly interesting for study as it may be a good model for changes in gene regulation early in the angiogenic "switch" mechanism and we are presently testing it for its responsiveness to changes in eIF4E that may "induce" the angiogenic phenotype.

VEGF was also variable in its expression between parental cell lines. We saw no evidence for alterations in VEGF isoform useage in response to eIF4E levels from western blotting, but in all cell lines tested, the total levels of secreted VEGF protein were modulated by eIF4E. An example of this in the PC3 cell line is presented in **Table 3**. As expected from the relative levels of eIF4E and VEGF isoforms, less secreted VEGF was detected in DU145 cells than in ALVA101 cells. In LNCaP cells, very little VEGF protein was detected by either western blotting or by ELISA, even though eIF4E levels are relatively high,

suggesting that the LNCaP cells might have a transcriptional suppression of VEGF messages that result in low levels of VEGF despite relatively high eIF4E.

Task 5: *In vivo* studies of retrovirus-mediated eIF4E antisense RNA on vascularization of tumors.
Timeline: Approximately 12 months to complete 2 sets of experiments (+/- cellulose sponges) using all 3 cell lines.
Milestones: Observation of differences in tumor size and latency depending on the cell line and viral construct used.
Animals used for this project: Approximately 320 mice.

Progress report for Task 5:

The animal studies were pushed back to begin in year 2 because of technical difficulties with maximal modulation of eIF4E levels. These problems have been largely resolved with the use of morpholine oligos and the herpes virus for eIF4E antisense delivery in vivo. The animal studies, including all work with the LuCaP23.1 cells, as well as the "sponge" experiments with all cell lines will begin in March 2000 and run through August 2000.

Task 6: *In vitro* analysis of the *in vivo* studies.
Timeline: Immediately after completion of each set of animal studies, we will begin the *in vitro* analyses. These should take approximately 10 months to complete, at least in part overlapping the *in vivo* studies.
Milestones: Completion of the immunohistochemical analyses of VEGF, FGF-2, and thrombospondin-1 in tumors and in the cellulose sponges. Completion of CD34 staining for microvessel density in tumors and in the cellulose sponges. Completion of statistical analysis of disease-free survival and multivariate analysis of the effect of eIF4E suppression on other biological markers.
Deliverables: At least 1 manuscript outlining the effect of eIF4E suppression *in vivo* on biological endpoints of vascularization and growth.

Progress report for Task 6:

Until the in vivo studies are completed, the proposed analyses and evaluation of the data cannot be initiated. Task 6 is planned for initiation by August 2000.

TABLES AND FIGURES

TABLE 1. COMPARISON OF THE EFFECT OF EIF4E ANTISENSE EXPRESSION ON THE GROWTH RATE IN 6 PROSTATE CELL LINES.

CELL LINE	% of parental or vector only cell growth of 4EAS transduced cells
PC3	60
PPC-1	60
DU145	60
ALVA 101	40
PZ-HPV-7	20
LNCaP	20

TABLE 2. COMPARISON OF THE EFFECT OF EIF4E ANTISENSE EXPRESSION ON SOFT AGAR COLONY FORMATION IN 6 PROSTATE CELL LINES.

CELL LINE	Number of soft agar colonies >50 cells- parental and vector only	Number of soft agar colonies >50 cells- 4EAS transduced
PC3	120	84
PPC-1	132	78
DU145	126	93
ALVA 101	133	56
PZ-HPV-7	3	2
LNCaP	118	26

Figure 1. Functional test of eIF4E. Human endothelial cells were plated in 6-well dishes and allowed to adhere for 6 hours, after which the media was changed to 2 mL of conditioned media collected from PC3 prostate cancer cells was added to each well and allowed to incubate for 72 hours. Capillary-like structures were observed in wells treated with conditioned media from PC3 cells transfected with an eIF4E sense construct (4E-S), as in panel a), but not in wells treated with conditioned media from PC3 parental cells as in panel b), or from cells transfected with an eIF4E antisense construct (4E-AS), as in panel c).

Functional test of eIF4E's effect on endothelial cells (HUVEC)

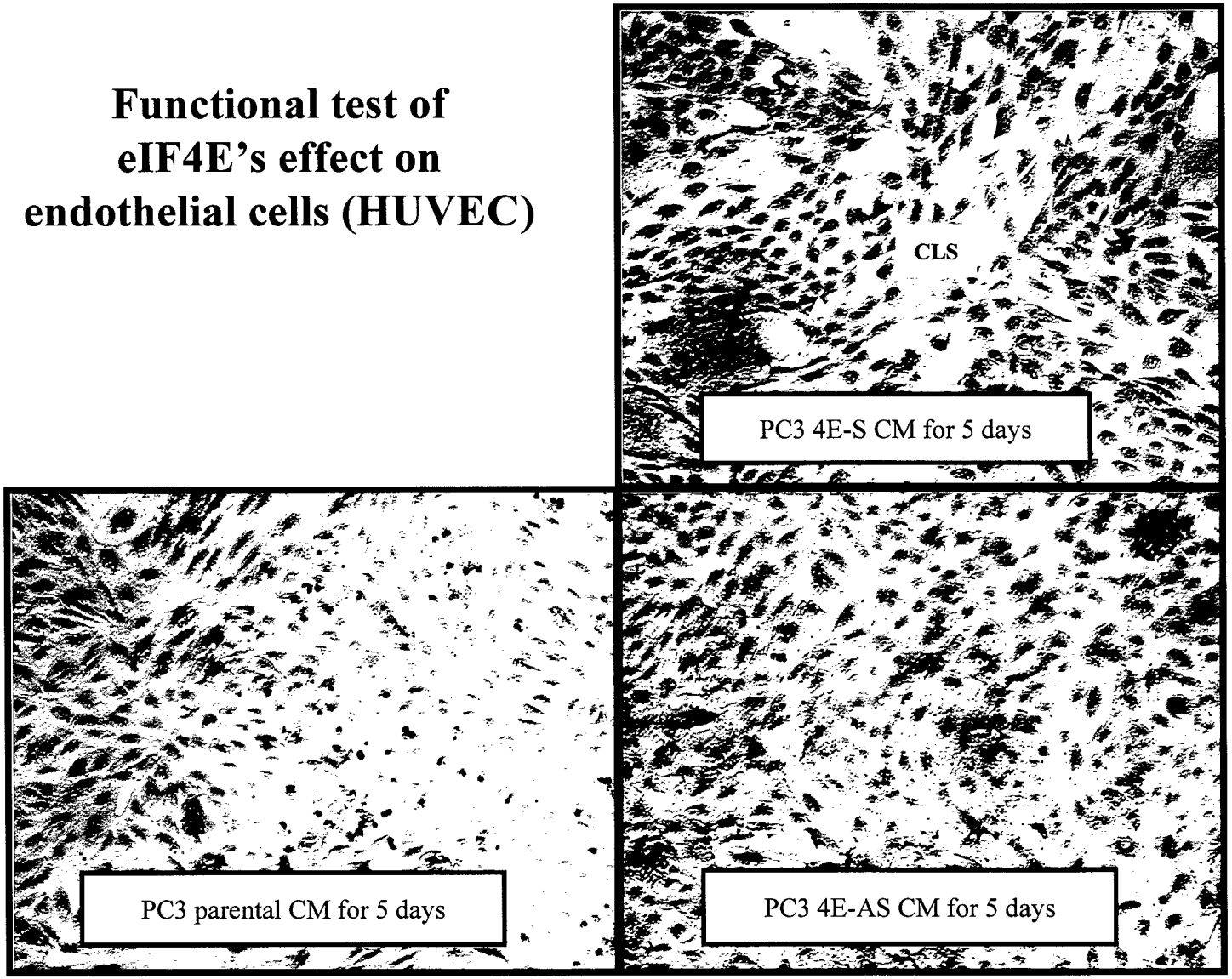

Figure 1

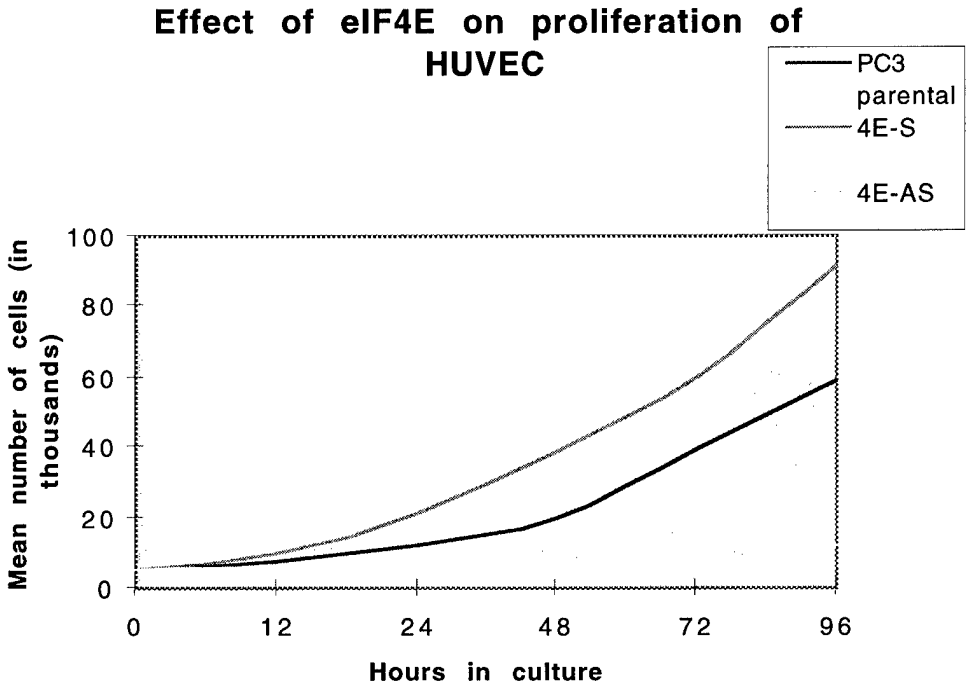

Figure 2. proliferation of HUVEC in response to culture in conditioned media from parental, eIF4E overexpressing (4E-S) or eIF4E suppressed cells (4E-AS).

Figure 3. Western blot illustrating levels of β-tubulin, eIF4E, FGF-2 isoforms, and VEGF isoforms in 5 prostate cancer cell lines and an HPV-E6/E7 immortalized normal prostate cell line. Relative expression of the 3 major isoforms of FGF-2 and VEGF were evaluated for differences from 20 μg of total protein in 6 prostate cell lines and compared with their level of eIF4E expression.

Figure 3

Prostate Cell Line Western Blot

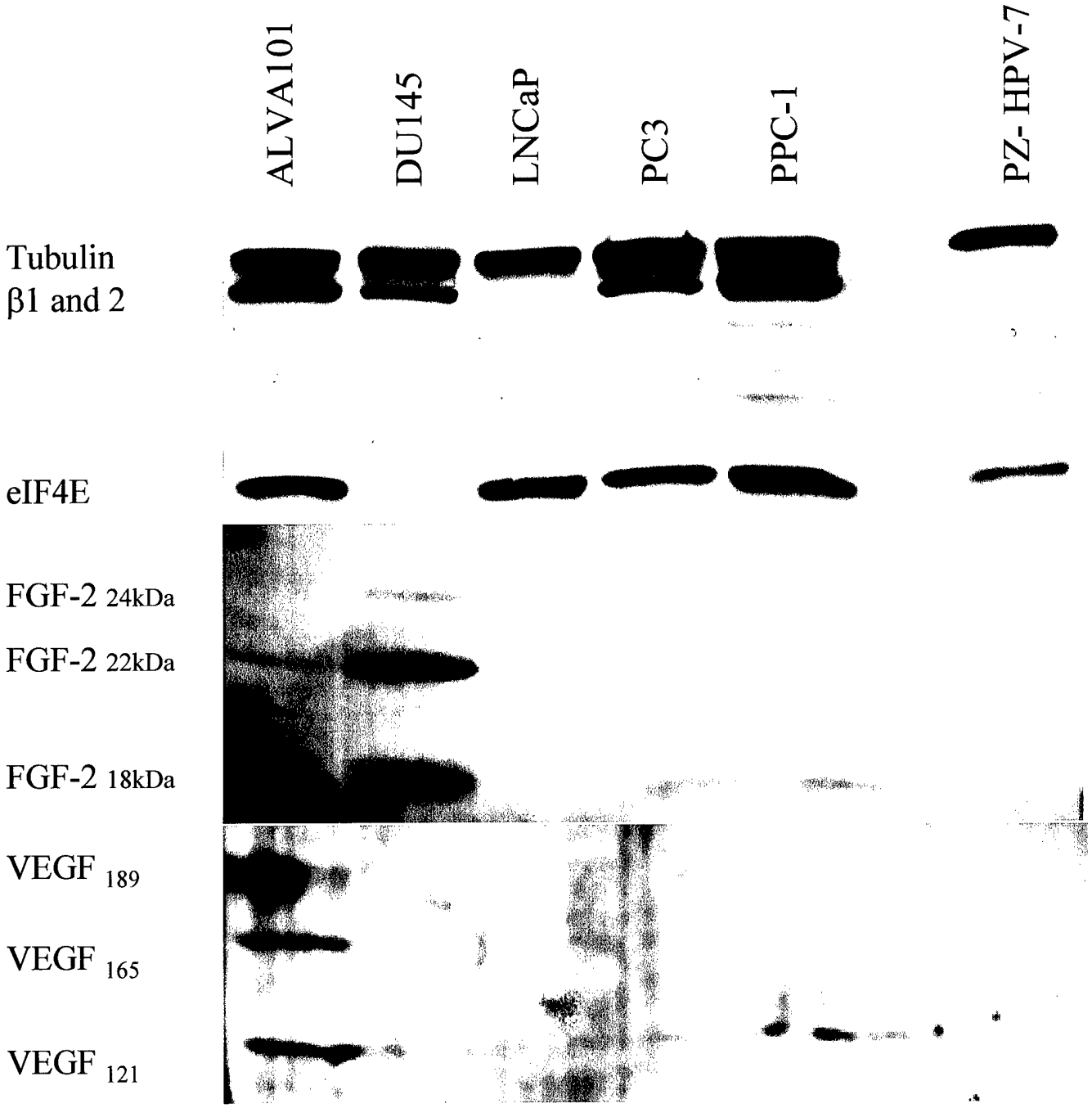

TABLE 3. LEVELS OF VEGF AND FGF-2 IN PARENTAL CELL LINES AND IN SUBLINES OF PC3 TRANSFECTED WITH AN INTEGRATING PLASMID FOR EITHER eIF4E SENSE OR ANTISENSE EXPRESSION.

Cell line	VEGF (pg/mL/10^6 cells)	FGF-2 (pg/mL/10^6 cells)
ALVA101	848	390
DU145	175	2.7
LNCap	undetectable	Not done
PC3	520	11.2
PC3 4E-S	1501	28.4
PC3 4E-AS	285	2.6

KEY RESEARCH ACCOMPLISHMENTS

- completion of "proof of principle" experiments illustrating the effect of eIF4E on tumor growth, expression of key angiogenic growth factors, and their functional effect on endothelial cell proliferation and differentiation

- establishment of 12 eIF4E expression sublines (6 overexpressing and 6 suppressed) of 6 prostate cell lines, including ALVA101, PC3, PPC-1, DU145, LNCaP, and PZ-HPV-7.

- development of new viral vectors and non-viral approaches to delivery of the eIF4E antisense construct

- manuscripts in preparation scheduled for submission in March and April, 2000

- accepted poster presentations based on this work

REPORTABLE OUTCOMES

Manuscripts in preparation:

James A. Eastham, Keith Tyler, Chris Stage, Nathan Goodyear, Elizabeth V. Mire, B. Jill Williams, "The influence of eIF4E on angiogenesis in prostate cancer from African-American men", expected to be submitted to American Journal of Pathology March, 2000.

Stephanie A. Cross, R. Ellen Friday, James A. Eastham, James A. Cardelli, B. Jill Williams, "Novel regulation of angiogenesis and metastasis: implications for prostate cancer in African-American men", expected to be submitted to Cancer Research, April, 2000.

B. Jill Williams, R. Ellen Friday, Peggy S. Carter, Stephanie A. Cross, James A. Eastham, "Regulation of prostate tumor angiogenesis by the translation initiation factor eIF4E: an antisense approach to anti-angiogenic therapy", expected to be submitted to Clinical Cancer Research, May, 2000.

Invited talks:

"Novel genes influencing prostate tumor angiogenesis", The Prostate Centre at Vancouver General Hospital, Vancouver, BC, September 10, 1999

"Novel regulation of prostate tumor angiogenesis and metastasis", M. D. Anderson Cancer Center, to be presented March 7, 2000.

Posters presented:

eIF4E Antisense RNA Modulation of Angiogenesis in Prostate Tumors: A Particularly Attractive Gene Therapy for African-American Men B. Jill Williams, Elizabeth V. Mire, Peggy S. Carter, Ellen Friday, James A. Eastham, Departments of Urology, Biochemistry and Molecular Biology, and the Feist-Weiller Cancer Center, Ray A. Barlow Symposium, LSU Health Sciences Center, Jan 29, 1999

Variable expression of FGF-2 and VEGF in prostate cancer cell lines that overexpress eIF4E. Peggy Carter, Elizabeth Mire, and Jill Williams. Departments of Urology, Biochemistry and Molecular Biology, and Feist -Weiller Cancer Center, Ray A. Barlow Symposium, LSU Health Sciences Center, Jan 29, 1999

Differential Gene Expression In Prostate Cell Lines Ellen Friday and B. Jill Williams, Departments of Urology, Biochemistry and Molecular Biology, and Feist -Weiller Cancer Center, Ray A. Barlow Symposium, LSU Health Sciences Center, Jan 29, 1999

Cultured Human Umbilical Vein Endothelial Cells Form Capillary-Like Structures In Response To eIF4E Expression Elizabeth V. Mire and B. Jill Williams, Departments of Urology, Biochemistry and Molecular Biology, and Feist -Weiller Cancer Center, Ray A. Barlow Symposium, LSU Health Sciences Center, Jan 29, 1999

B. Jill Williams, Keith Tyler, Chris Stage, Elizabeth V. Mire, James A. Eastham,

The influence of eIF4E on angiogenesis in prostate tumors from African-American men, J. Urol., 140(6):505A. American Urological Association, May 1-5, 1999

Suppression of eIF4E by a morpholino antisense oligonucleotide decreases expression of VEGF in the PC3 prostate cancer cell line. B. Jill Williams, Departments of Urology and Biochemistry/Molecular Biology and the Feist-Weiller Cancer Center, LSU Health Sciences Center, Shreveport, LA, to be presented at the "Advances in Human Breast and Prostate Cancer" Keystone Symposium, Tahoe, NV, March 18-24, 2000.

Novel Regulation of angiogenesis in prostate cancer, SA Cross, RE Friday, BJ Williams, LSU Health Sciences Center, Shreveport, LA, to be presented by SA Cross at the "Advances in Human Breast and Prostate Cancer" Keystone Symposium, Tahoe, NV, March 18-24, 2000.

Novel Regulation of angiogenesis in prostate cancer, SA Cross, RE Friday, BJ Williams, LSU Health Sciences Center, Shreveport, LA, to be presented by Dr. Williams at the annual meeting of the American Association for Cancer Research, San Fransisco, CA, April 1-5, 2000.

Funding obtained based on work supported by this award:

B. Jill Williams, awarded the Edwin W. Beer Fellowship in Urology, New York Academy of Medicine, "The Translation Initiation Factor eIF4E: A Novel Mediator of Prostate Tumor Growth and Angiogenesis", July 1999-June 2001, $75,000 total (salary support only), $37,500 annual direct cost.

Funding applications planned based on this award:
DOD Phase II

Student and Postdoctoral Training in support of this grant (no salary received from this award unless specified):

1. Peggy S. Carter, Ph.D., September 1998-October 1999, Cancer Center funding
2. Stephanie A. Cross, M.S. candidate, June 1999-present, Cancer Center funding
3. Chris A. Stage, MI student, supported by an American Foundation for Urologic Disease summer medical student fellowship
4. Keith Tyler, undergraduate student, supported by NCI minority supplement
5. Nathan Goodyear, MI student, supported by departmental funds
6. Marta Jarquin-Pardo, rotation graduate student, supported by departmental funds
7. Laura McGhee, rotation graduate student, supported by departmental funds

CONCLUSIONS

The completed work has contributed to both basic research knowledge and to clinical investigation of a novel experimental therapeutic. Our studies have demonstrated that the translation initiation machinery, specifically eIF4E, is frequently dysregulated in prostate cancer and that alone may provide a growth advantage to the tumor through increased angiogenesis. From previous studies of our own as well as others, we believe eIF4E is a particularly interesting and important target as it controls expression of a number of genes simultaneously, many of these genes being important angiogenic growth factors. Our studies have generated a collection of prostate cell lines with varying levels of eIF4E in order to determine the effect on tumor cell growth and angiogenesis both in vitro and in vivo. From these studies, we can see clearly that through modulation of total levels of both VEGF and FGF-2, eIF4E can increase cell growth, promote loss of contact inhibition, and increase proliferation and differentiation of endothelial cells, while suppression of eIF4E reverses these growth parameters. These results would suggest that our *in vivo* studies planned for year 2 will illustrate the effectiveness of eIF4E suppression in decreasing both tumor growth and its angiogenic phenotype.

We found that while prostate tumors have a high frequency of eIF4E elevation, and this was related to the increase in both VEGF and FGF-2, the molecular mechanism underlying this correlation was different than seen by other investigators in a breast cancer cell line. The mechanism used in prostate cancer appears to be a general increase in translation of all isoforms of either VEGF or FGF-2 rather than a switch to a more highly secreted form as suggested in breast cancer. Another of our goals was to determine if p53 status would influence the effect of changes in eIF4E levels. Since we found essentially the same results in all of our cell lines tested, whether p53 null, mutant, or wild-type, eIF4E appears to have a stronger effect on cell growth and expression of angiogenic growth factors. This is encouraging news for the development of eIF4E suppression as a viable gene therapy as it may be effective in virtually all prostate tumors, irrespective of p53 status.

While we have not completed additional animal experiments, our preliminary studies suggested that tumors with eIF4E levels suppressed by a targeted anti-eIF4E antisense oligo would not grow in nude mice. In addition, eIF4E-suppressed tumors grown in a polyurethane sponge support would not promote infiltration of blood vessels into the sponges. We are confident given the results from our expanded in vitro studies that we will reproduce these results with other cell lines having different genetic backgrounds.

I outlined recommendations for changes to the work for each task above. Briefly, we would suggest 1) development of a herpes virus-mediated delivery system rather than the retrovirus proposed and for the development of the synthetic morpholine oligonucleotide as a non-viral alternative. Both of these have been much more successful in our hands than our plasmid system or the retrovirus. In addition, we would suggest 2) expansion of our investigation to include the effect of TIE-1, Kdr, and VEGF-C in prostate tumor growth and progression. All of these were discovered to be regulated by changes in eIF4E levels and each of these genes has a clear link to the angiogenic phenotype both in vitro and in vivo.

The most significant outcome of the studies proposed in this award is the development of anti-eIF4E as a viable gene therapy for human prostate cancer. We have completed in vitro "proof of principle" studies that support our hypothesis that anti-eIF4E will suppress tumor growth and angiogenesis. Our animal studies to be completed in year 2 will hopefully support this hypothesis as well. Our goal is to prepare a limited clinical trial based on our studies.

REFERENCES

1. DeBenedetti, A., *et al.*, *CHO cells transformed by the translation initiation factor eIF4E display increased c-myc expression but require overexpression of max for tumorigenicity.* Mol Cell Diff, 1994. **2**: p. 347-371.
2. Bouck, N., *p53 and angiogenesis.* Biochimica et Biophysica Acta, 1996. **1287**: p. 63-66.
3. Grossfeld, G.D., *et al.*, *Thrombospondin-1 expression in bladder cancer: association with p53 alterations, tumor angiogenesis, and tumor progression.* Journal of the National Cancer Institute, 1997. **89**(3): p. 219-227.
4. Brawer, M.K., *et al.*, *Predictors of pathologic stage in prostate carcinoma: the role of neovascularity.* Cancer, 1994. **73**(3): p. 678-687.
5. Silberman, M.A., *et al.*, *Tumor angiogenesis correlates with progression after radical prostatectomy but not with pathologic stage in Gleason sum 5 to 7 adenocarcinoma of the prostate.* Cancer, 1997. **79**(4): p. 772-779.
6. Kevil, C., *et al.*, *Translational enhancement of FGF-2 by eIF-4 factors, and alternate utilization of CUG and AUG codons for translation initiation.* Oncogene, 1995. **11**: p. 2339-2348.